Eur J Med Chem (1991) 26, 273–278

Synthesis and cytotoxic activity on islets of Langerhans of benzamide thiosemicarbazone derivatives

M Campana[1,2], C Laborie[2], G Barbier[1], R Assan[2], R Milcent[1]*

[1]*Laboratoire de Chimie Organique Médicale, Unité de Recherche Chimie et Pharmacologie;*
[2]*Laboratoire de Diabétologie expérimentale et clinique,*
Faculté de Médecine Xavier-Bichat, Université Paris 7, 16, rue Henri Huchard, 75018 Paris, France

(Received 2 May 1990; accepted 2 October 1990)

Summary — Eleven 1-(4-substituted α-arylaminobenzylidene)thiosemicarbazides **1** and the related semicarbazones **2** were synthesized and tested *in vitro* for their inhibitory effects on the islets of Langerhans. Only the thiosemicarbazones **1** suppressed the insulin and glucagon secretions while the somatostatin release persisted. The 1-(α-anilino-4-methylbenzylidene)thiosemicarbazide **1f** was the most potent suppressor of insulin release and lysed the islet β cells. Zinc sulfate protected islets from the suppressive and toxic effects of **1f**. These compounds **1** could be potential drugs for the treatment of insulinomas.

Résumé — Synthèse de dérivés de la thiosemicarbazone du benzamide et activité cytotoxique sur les îlots de Langerhans. *Onze 1-(α-arylaminobenzylidène)thiosemicarbazides **1** ainsi que les semicarbazones homologues **2** ont été synthétisées et testées pour leurs propriétés inhibitrices sur les sécrétions des îlots de Langerhans. Seules les thiosemicarbazones **1** inhibent la sécrétion d'insuline et de glucagon: celle de somatostatine persiste. La molécule la plus active est la 1-(α-anilino-4-méthylbenzylidène)thiosemicarbazide **1f**. La présence de sulfate de zinc supprime les effets inhibiteurs et cytotoxiques de **1f**. De tels composés pourraient avoir une application dans le traitement des insulinomes.*

benzamide thiosemicarbazone derivatives / benzamide semicarbazone derivatives / Langerhans islets

Thiosemicarbazones are known for their antitumoral [1], antiviral [2], antiparasitic [3], antiinflammatory [4] and tuberculostatic [5] properties. Their activities have often been related to their ability to form complexes with transition metal ions [6]. Diabetogenic agents such as dithizone, oxine, quinaldic acid or quinoline derivatives appear to act *via* chelation of zinc in the pancreatic β cells [7]. Up to now, treatment of surgically unextirpable insulinomas is an unresolved problem. Use of specific cytotoxic drugs of β cells of islets of Langerhans, such as streptozotocine or pentamidine, has been proposed to palliate this difficulty [8], with, as yet, unsatisfactory results.

In order to develop new cytotoxic agents (diabetogenic) as potential therapeutic drugs, the effective zinc chelating benzamide thiosemicarbazone derivatives, 1-(4-substituted α-arylaminobenzylidene)thiosemicarbazides **1**, the related semicarbazones **2**, the semicarbazones **3** and thiosemicarbazones **4** were synthesized. The inhibitory activities of these compounds against insulin as well as glucagon and somatostatin secretions were evaluated *in vitro* on isolated rat islets of Langerhans.

*Correspondence and reprints

Chemistry

The thiosemicarbazones **1a–h** and semicarbazones **2a–h** (table I) have been prepared by treatment of amidrazones **5a–h** by phenylisothiocyanide [9] or phenylisocyanide, respectively, in chloroform at 0°C (scheme 1).

Amidrazones **5** were formed, apart from traces of dihydrotetrazines as by-products, by the action of hydrazine hydrate on the corresponding thioamides **6** [10]. These thioamides were obtained by sulfuration of the corresponding amides with phosphorous pentasulfide [11] in pyridine as solvent.

Thiosemicarbazones **1i–k** and the semicarbazone **2i** (table I) were synthesized by reaction of methyl thioimidates **7a–c** with thiosemicarbazide or semicarbazide, respectively [13]. Finally, arylimidate hydro-

chlorides **8a**, **b** were reacted with semicarbazide in the presence of triethylamine to give the semicarbazones **3a**, **b** [14] and with thiosemicarbazide in DMF to give the thiosemicarbazones **4a**, **b** [15].

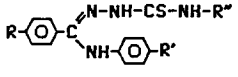
Physico-chemical data were in agreement with the proposed structures.

Results and Discussion

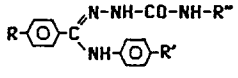
The 24 compounds **1**, **2**, **3**, **4** were evaluated *in vitro* for their ability to suppress the insulin release induced by arginine + theophylline in isolated rat islets of Langerhans. All thiosemicarbazones **1** inhibited the insulin release (table II). However, only the 4 thiosemicarbazones **1f**, **g**, **j**, **k** are significantly active in this series (IC_{50} < 20 μM). The related compounds **2**, **3** and **4** did not affect the β cell response (IC_{50} > 100 μM). That is, the replacement of either the thiocarbonyl group by a carbonyl one (**1** *vs* **2** or **3**) or the anilino group by an ethoxy one (**1** *vs* **4**) leads to inactive compounds.

For thiosemicarbazones **1**, substitution at N-4 by a phenyl group (substituent R") did not markedly influence the activity (**1a**, **f**, **h** *vs* **1i–k**). Likewise, in compounds **1**, the substitution by R' = OMe, Me or Cl (**1b**, **1c**, **1d**, respectively) did not significantly modify

Table I. 1-(4-substituted α-arylaminobenzylidene)thiosemicarbazides **1**.



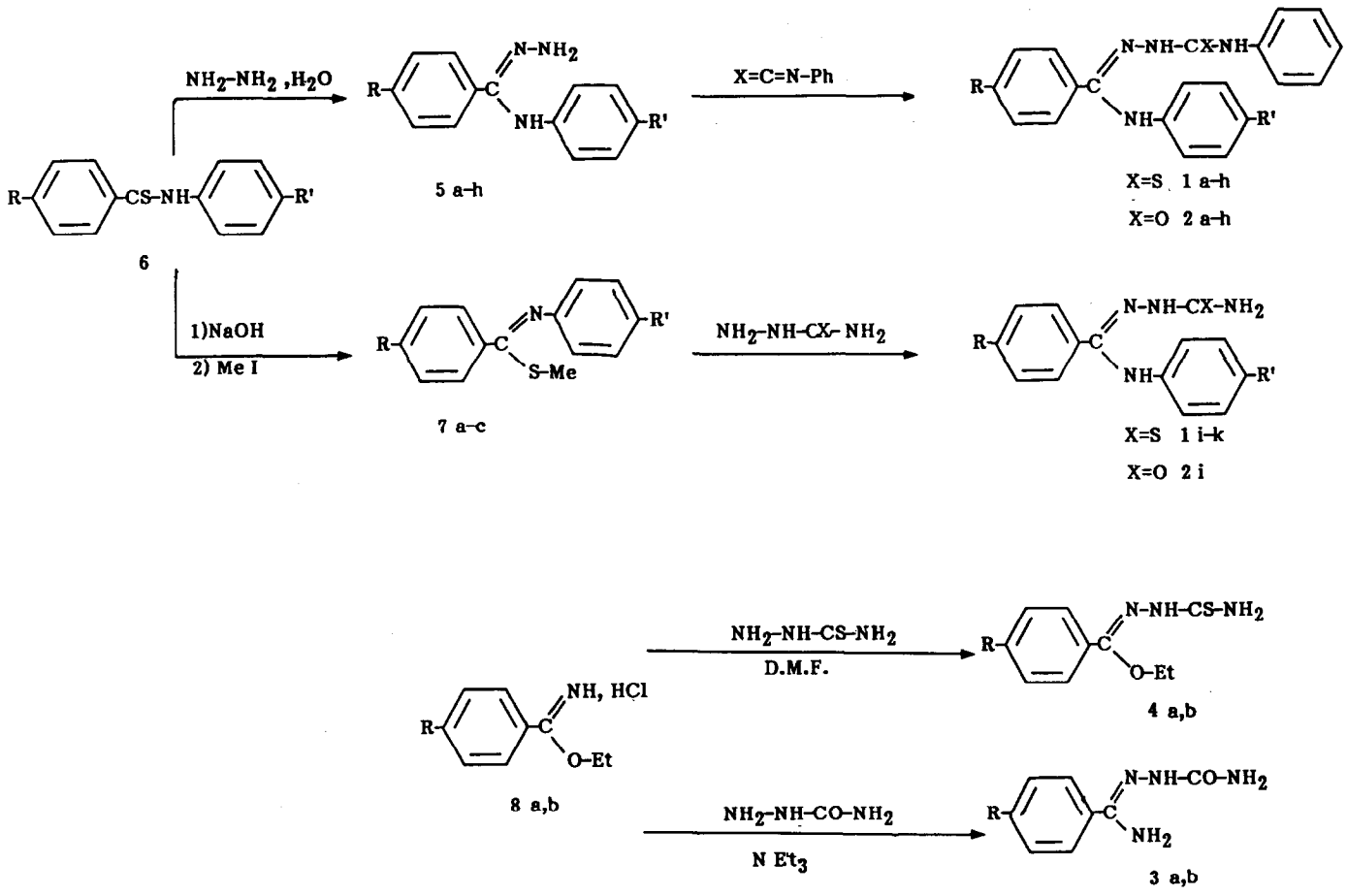
1	*R*	*R'*	*R"*	*mp*[a] *(°C)*	*Yield (%)*[b]	*Formula*	*PMR δ (ppm)*
a	H	H	Ph	153[c]	50	$C_{20}H_{18}N_4S$	6.5–7.8 (m, 15H), 9.1 (s, 1H), 9.9 (s, 1H), 10.6 (s, 1H)
b	H	MeO	Ph	142	60	$C_{21}H_{20}N_4OS$	3.65 (s, 3H), 6.7 (s, 4H), 7.15–7.85 (m, 10H), 8.9 (s, 1H), 9.75 (s, 1H), 10.4 (s, 1H)
c	H	Me	Ph	146[d]	55	$C_{21}H_{20}N_4S$	2.2 (s, 3H), 6.6 (d, 2H), 7 (d, 2H), 7.15–7.8 (m, 10H), 9 (s, 1H), 9.8 (s, 1H), 10.45 (s, 1H)
d	H	Cl	Ph	162	71	$C_{20}H_{17}ClN_4S$	6.65 (d, 2H), 7.1–7.5 (m, 8H), 7.65 (d, 4H), 9.1 (s, 1H), 9.85 (s, 1H), 10.4 (s, 1H)
e	MeO	H	Ph	174	62	$C_{21}H_{20}N_4OS$	3.8 (s, 3H), 6.5–7.8 (m, 14H), 9 (s, 1H), 9.9 (s, 1H), 10.45 (s, 1H)
f	Me	H	Ph	161	63	$C_{21}H_{20}N_4S$	2.3 (s, 3H), 6.65–7.8 (m, 14H), 9 (s, 1H), 9.85 (s, 1H), 10.5 (s, 1H)
g	Cl	H	Ph	152–3	52	$C_{20}H_{17}ClN_4S$	6.7 (d, 2H), 6.85–7.5 (m, 8H), 7.65 and 7.7 (2d, 4H), 9.05 (s, 1H), 9.9 (s, 1H), 10.45 (s, 1H)
h	NO_2	H	Ph	198	30	$C_{20}H_{17}N_5O_2S$	6.5–8.3 (m, 14H), 9.25 (s, 1H), 10.15 (s, 1H), 10.8 (s, 1H)
i	H	H	H	212[e]	60	$C_{14}H_{14}N_4S$	6.4–7.7 (m, 11H), 8.1 (bs, 1H), 8.8 (s, 1H), 10 (s, 1H)
j	Me	H	H	184	65	$C_{15}H_{16}N_4S$	2.25 (s, 3H), 6.6 (d, 2H), 6.8–7.3 (m, 5H), 7.5 (d, 2H), 7.65 (bs, 1H), 8.15 (bs, 1H), 8.85 (s, 1H), 10.05 (s, 1H)
k	NO_2	H	H	220	87	$C_{14}H_{13}N_5O_2S$	6.45–7.3 (m, 5H), 7.6–8.3 (m, 6H), 8.95 (s, 1H), 10.25 (s, 1H)



1-(4-substituted α-arylaminobenzylidene)semicarbazides **2**.

2	*R*	*R'*	*R"*	*mp*[a] *(°C)*	*Yield (%)*[b]	*Formula*	*PMR δ (ppm)*
a	H	H	Ph	206	35	$C_{20}H_{18}N_4O$	6.5–7.8 (m, 15H), 8.5 (s, 1H), 8.9 (s, 1H), 9.55 (s, 1H)
b	H	MeO	Ph	214	72	$C_{21}H_{20}N_4O_2$	3.65 (s, 3H), 6.5–7.6 (m, 14H), 8.15 (s, 1H), 8.6 (s, 1H), 9.25 (s, 1H)
c	H	Me	Ph	232	75	$C_{21}H_{20}N_4O$	2.15 (s, 3H), 6.5 (d, 2H), 6.85–7.75 (m, 12H), 8.3 (s, 1H), 8.8 (s, 1H), 9.35 (s, 1H)
d	H	Cl	Ph	242	65	$C_{20}H_{17}ClN_4O$	6.65 (d, 2H), 6.9–7.5 (m, 8H), 7.6 (d, 4H), 8.5 (s, 1H), 8.8 (s, 1H), 9.4 (s, 1H)
e	MeO	H	Ph	208	30	$C_{21}H_{20}N_4O_2$	3.8 (s, 3H), 6.6–7.9 (m, 14H), 8.5 (s, 1H), 8.95 (s, 1H), 9.45 (s, 1H)
f	Me	H	Ph	208	37	$C_{21}H_{20}N_4O$	2.3 (s, 3H), 6.65 (d, 2H), 6.75–7.75 (m, 12H), 8.35 (s, 1H), 8.8 (s, 1H), 9.35 (s, 1H)
g	Cl	H	Ph	229	46	$C_{20}H_{17}ClN_4O$	6.5 (d, 2H), 6.7–7.6 (m, 12H), 8.35 (s, 1H), 8.75 (s, 1H), 9.3 (s, 1H)
h	NO_2	H	Ph	220	22	$C_{20}H_{17}N_5O_3$	6.6 (d, 2H), 6.7–7.9 (m, 8H), 7.95 and 8.25 (2d, 4H), 8.6 (s, 1H), 9 (s, 1H), 9.75 (s, 1H)
i	H	H	H	218	83	$C_{14}H_{14}N_4O$	6.3–7.65 (m, 12H), 8.25 (s, 1H), 8.8 (s, 1H)

[a]Recristallization solvent: ethanol. [b]Yields were not optimized. [c]Lit [9] mp = 150–151°C. [d]Lit [9] mp = 141°C. [e]Lit [12] mp non mentioned.

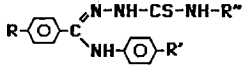


Scheme 1.

the activity of **1a** (R' = H). On the contrary, significant variations of IC_{50} values were observed with the nature of the R substituent but no correlation has been shown between these values and the electronic contribution of the R group.

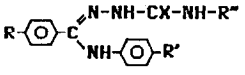
In parallel to these studies, we have examined the effects of thiosemicarbazones **1**, in particular, on both glucagon and somatostatin release induced by arginine + theophylline (table III) in order to observe an eventual intrainsular specificity of **1**. Tested at 10^{-4} M, all thiosemicarbazones **1** inhibited the release of glucagon by the islet A cells, but not that of somatostatin by the islet D cells. Compounds **2**, **3** and **4** were without effects. The thiosemicarbazide did not suppress the β, A and D cells responses to arginine + theophylline. The cytotoxic activity was determined by a Trypan blue exclusion test. One min following addition of the isotonic Trypan blue solution, the dark blue islets were distinguishable from the bright white intact islets. Nine percent of the islets (*vs* 86% for control islets) were unstained after 18 h in the presence of 10^{-4} M **1f**.

Table II. Influence of thiosemicarbazones **1** on the β cell response from rat islets incubated *in vitro*. IC_{50}, compound concentration that produces 50% of insulin secretory response to arginine + theophylline

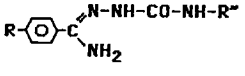


1	*R*	*R'*	*R''*	IC_{50} (*μM*)
a	H	H	Ph	59 ± 13
b	H	MeO	Ph	52 ± 14
c	H	Me	Ph	60 ± 9
d	H	Cl	Ph	71 ± 21
e	MeO	H	Ph	70 ± 10
f	Me	H	Ph	3 ± 1
g	Cl	H	Ph	10 ± 5
h	NO_2	H	Ph	46 ± 7
i	H	H	H	52 ± 8
j	Me	H	H	14 ± 3
k	NO_2	H	H	8 ± 2

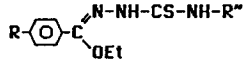
Table III. Influence of 10^{-4} M thiosemicarbazones **1** and **4** and semicarbazones **2** and **3** on the A and D cells responses from rat islets incubated *in vitro*. The 100% response corresponded to control rat islets, incubated for 18 h in basal medium and stimulated by 20 mM arginine + 5 mM theophylline for 30 min. Results are expressed as mean ± SEM.



N°	*X*	*R*	*R'*	*R''*	*Glucagon (%)[a]*	*Somatostatin (%)[b]*
1a	S	H	H	Ph	15 ± 6*	115 ± 13
1b	S	H	MeO	Ph	7 ± 2*	90 ± 6
1c	S	H	Me	Ph	11 ± 5*	80 ± 5
1d	S	H	Cl	Ph	11 ± 3*	79 ± 14
1f	S	Me	H	Ph	8 ± 1*	80 ± 5
1g	S	Cl	H	Ph	5 ± 1*	85 ± 9
1h	S	NO_2	H	Ph	14 ± 5*	112 ± 16
1k	S	NO_2	H	H	13 ± 2*	115 ± 8
2c	O	H	Me	Ph	95 ± 6	90 ± 10
2d	O	H	Cl	Ph	78 ± 8	127 ± 16
Control islets					100 ± 13	100 ± 6



3	*R*	*R''*	*Glucagon (%)[a]*	*Somatostatin (%)[b]*
b	Me	H	–	91 ± 7



4	*R*	*R''*	*Glucagon (%)[a]*	*Somatostatin (%)[b]*
a	H	H	84 ± 11	125 ± 8
b	Me	H	–	113 ± 16
Thiosemicarbazide			125 ± 22	91 ± 9

*$P < 0.001$ statistical differences between control and test islets. [a]Percent of control glucagon secretory response to arginine + theophylline. [b]Percent of control somatostatin secretory response to arginine + theophylline.

Some extrainsular cell lines (fetal rat fibroblasts, hepatocytes from Wistar rats, Madin Darby Canine Kidney MDCK) appeared to be less sensitive than β cells to the thiosemicarbazone **1f**, the most potent inhibitor in the series (results to be published).

A protective effect of zinc sulfate on β and A cells function in the presence of the thiosemicarbazone **1f** was detected (table IV) and the Trypan blue exclusion correlated this result. Zinc ions could complex the compound **1f** before it suppressed β and A cell functions. The study of complexes between zinc ions and ketone thiosemicarbazones were reported [16]. Compounds **1a** and **1i** were also reported as being able to chelate molybdenum (V) [12]. The formation of a chelate between **1f** and zinc ions was shown by the comparison of PMR spectra (in DMSO–d_6) of compound **1f** in the absence or presence of zinc ions. On the contrary, under the same conditions, the PMR spectra of the parent semicarbazone **2f** was not modified.

Zinc plays an important role in insulin biosynthesis, storage and secretion [17, 18] and in glucagon storage by stabilizing the conformation of the glucagon molecule [19]. Other substances which chelate zinc ions have been shown to be diabetogenic. Thus, the induction of chemical diabetes by diphenylthiocarbazone, for example, is accompanied by a loss of histochemically detectable zinc from pancreatic β cells [20].

Elsewhere, the 2-formylthiosemicarbazones has formed strong coordination complexes, often intensi-

Table IV. Protective effect of Zn^{2+} against the β and A cells with regard to toxicity of compound **1f**. The 100% response corresponded to control rat islets, incubated for 18 h in basal medium and then stimulated by 20 mM arginine + 5 mM theophylline for 30 min. Test islets were exposed to 10^{-4} M **1f** and to 10^{-4} M salts. Results are expressed as mean ± SEM.

	Insulin (%)[a]	*Glucagon (%)[b]*	*Percent of unstained islets (Trypan blue)*
Control islets	100 ± 4	100 ± 13	86
1f	13 ± 2***	8 ± 1***	9
1f + Zn^{2+}	129 ± 20	121 ± 34	89
1f + Ca^{2+}	31 ± 3**	17 ± 1***	31
1f + Mg^{2+}	8 ± 1***	–	10
1f + Fe^{2+}	17 ± 3***	–	4
1f + Fe^{3+}	16 ± 2***	–	6
Islets + Zn^{2+}	77 ± 1*	197 ± 65	82
Islets + Ca^{2+}	86 ± 10	125 ± 16	100
Islets + Mg^{2+}	128 ± 4	–	82
Islets + Fe^{2+}	82 ± 6	–	81
Islets + Fe^{3+}	83 ± 5	–	87

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$: statistical differences between control and test islets; [a]percent of control insulin secretory response to arginine + theophylline; [b]percent of control glucagon secretory response to arginine + theophylline.

vely colored, with divalent ions (Fe, Co, Ni, Cu, Zn) but did not coordinate Mg and Ca [21]. With the compound **1f** (at 10^{-4} M for 18 h) the suppression of the β and A cells responses to arginine + theophylline was not affected by the presence of 10^{-4} M calcium chloride or magnesium sulfate in an incubation medium. In the same manner, neither the presence of iron (II) sulfate, nor that of iron (III) chloride protected the β cell function from **19f** effects. The Trypan blue exclusion correlated this result (table IV).

The antineoplastic activity of thiosemicarbazide derivatives, which was elucidated with 1-formylisoquinoline thiosemicarbazone, required metal binding potential and the capacity to inhibit the synthesis of DNA [22].

Experimental protocols

Chemistry

Melting points (uncorrected) were determined by using a Buchi oil heated apparatus. IR spectra were recorded as KBr disks with a Perkin Elmer 1310 spectrophotometer. PMR spectra were recorded on a Brucker WP 80 spectrometer in DMSO–d_6 with tetramethylsilane as the internal reference. Analytical results obtained for C, H, N of new compounds were within ± 0.4% of the calculated values.

*1-(4-Substituted α-arylaminobenzylidene)-4-phenylthiosemicarbazides **1a–h***

Phenylisothiocyanate (0.55 g, 4 mmol) was added at 0°C to a solution of benzamidrazone **5** (4 mmol) in dry chloroform (20 ml). The precipitated compounds **1** were filtered and recrystallized from ethanol (table I). IR (KBr) cm^{-1}: 3350–3320, 3300–3250, 3190–3120 (NH).

*1-(4-Substituted α-anilinobenzylidene)thiosemicarbazides **1i–k***

To a solution of thioimidate **7** (8 mmol) in dry DMF (25 ml) was added thiosemicarbazide (0.91 g, 10 mmol). The reaction mixture was stirred at room temperature for 5 d and poured into cold water. The precipitated compounds **1** were filtered and recrystallized from ethanol (table I). IR (KBr) cm^{-1}: 3400, 3330–3320, 3280–3260, 3160–3140 (NH and NH_2).

*1-(4-Substituted α-arylaminobenzylidene)-4-phenylsemicarbazides **2a–h***

Phenylisocyanate (0.47 g, 4 mmol) was added at 0°C to a solution of benzamidrazone **5** (4 mmol) in dry chloroform (20 ml). The precipitated compounds **2** were filtered and recrystallized from ethanol (table I). IR (KBr) cm^{-1}: 3360, 3220–3200 (NH), 1670–1665 (CO).

*1-(α-Anilinobenzylidene)semicarbazide **2i***

To a solution of thioimidate **7a** (8 mmol) in dry DMF (15 ml) was added a solution of semicarbazide hydrochloride (1.11 g, 10 mmol) and triethylamine (10 mmol) in methanol (10 ml). The reaction mixture was stirred at room temperature for 3 d and poured into cold water. The precipitated compound **2i** was filtered and recrystallized from ethanol (table I). IR (KBr) cm^{-1}: 3340, 3190 (NH and NH_2), 1650 (CO).

*Amidrazones **5a–h***

Compounds **5a**, **c**, **h** [10], **5b**, **f** [23], **5d**, **e** [24] have been synthesized by a published method [10]. The new amidrazone **5g** has been prepared by the same method in 63% yield; mp = 91–92°C. IR (KBr) cm^{-1}: 3380, 3340 (NH and NH_2). PMR δ ppm: 6–7.6 (m). Anal $C_{13}H_{12}ClN_3$ (C, H, N).

*Methyl N-phenylthiobenzimidates **7a–c***

To a stirred solution of 5 mmol of the corresponding *N*-phenylthiobenzamide **6** (*R'* = H with *R* = H, Me or NO_2, respectively) in 1 N sodium hydroxyde ethanol (20 ml) methyl iodide (1.13 g, 8 mmol) was added at 0°C. Spontaneously, the product partially precipitated. Precipitation was completed by pouring the mixture into cold water. The precipitated product was filtered and recrystallized from ethanol to give **7a**, mp = 61°C (Lit [25] mp = 63–64°C). **7b** Yield: 0.9 g (75%), mp = 49°C. IR (KBr) cm^{-1}: 1610 (C=N). PMR δ ppm: 2.35 (s, 6H), 6.5–7.5 (m, 9H). Anal $C_{15}H_{15}NS$ (C, H, N). **7c** Yield: 1.18 g (92%), mp = 104°C. IR (KBr) cm^{-1}: 1610 (C=N). PMR δ ppm: 2.2–2.6 (m, 3H, *E* + *Z*), 6.7–8.4 (m, 9H). Anal $C_{14}H_{12}N_2O_2S$ (C, H, N).

*PMR spectra of **1f** and **2f** in the presence of zinc ions*

Spectra of compound **1f** were run with 3 molar ratios of **1f** relative to dry zinc chloride: 1/1, 2/1 and 4/1. A 219 mM reference solution of **1f** (158 mg, 0.44 mmol) in DMSO–d_6 (2 ml) was prepared. Then, 660, 560 and 700 µl of this solution were added to 19 mg (0.14 mmol), 8 mg (0.061 mmol) and 5 mg (0.038 mmol), respectively, of dry zinc chloride. The three NH singlets at 9 ppm (α-N), 9.85 ppm (N-2) and 10.45 ppm (N-4) were identified by comparison of spectra of compounds **1a–h**, **1i–k** and **4a**, **b** without zinc ions. An extinction was observed for the 9 and 10.45 ppm signals while the 9.85 ppm signal became a fairly broad singlet. This result is in agreement with a complex formation [22].

Spectra of compound **2f** (55 mg, 0.16 mmol) in DMSO–d_6 (0.5 ml) was run. When this solution was added to 21 mg (0.16 mmol) of dry zinc chloride, the 3 NH singlets at 8.35, 8.8 and 10.45 ppm of the PMR spectra were unaffected.

Biological assays

Functional studies

Pancreatic islets of Langerhans were isolated from non-fasted male Wistar rats (IFFA Credo, L'Arbresles, France) by using a collagenase method [26]. Islets were purified either by hand picking or by centrifugation on a density gradient method [27]. They were then cultured for 24 h at 37°C under an O_2/CO_2 (95%/5%) atmosphere in MEM (Minimum Eagle Medium, Gibco, Paris, France) supplemented with 0.814 mg/l non essential aminoacids, 1 mM sodium pyruvate, 2 mM glutamine, 10% inactivated fetal calf serum (FCS, Flow) and 100 µU/ml penicillin + 100 µg/ml streptomycin. This basal medium contained glucose at a concentration of 5 mM. All compounds (0.01 mmol) were dissolved in DMSO (1 ml) and diluted in basal medium. They were then transferred into microtitration plates (Beckton-Dickinson, Rockville, MD, USA). Five islets per well were exposed to compounds for 18 h at 10^{-4}, 2×10^{-5} or 10^{-6} M under an O_2/CO_2 (95%/5%) atmosphere.

At the end of the incubation period, the islets were washed twice and exposed for 30 min either to a basal medium (containing 5 mM glucose) or to a stimulatory medium containing 20 mM L(+) arginine hydrochloride and 5 mM theophylline. Supernatants were harvested and added to 4000 U/ml trasylol + 12 mg/ml EDTA and then kept frozen at – 20°C until hormone determination.

Control experiments were run in parallel in the presence of DMSO (1% v/v), which did not affect the cell responses to arginine + theophylline. Similar experiments were conducted for compound **1f** in the presence of zinc sulfate, magnesium sulfate or calcium chloride during incubation.

Insulin, glucagon and somatostatin were determined by radioimmunoassay [28] using charcoal for the separation of free and bound hormone [29]. Purified rat insulin (Novo, Copenhagen, Denmark), pork glucagon (Novo) and synthetic cyclic somatostatin (Clin Midi, Montpellier, France) were used as standards. The glucagon antiserum was the 30 K serum from R Unger (Dallas, Tex, USA) and that used for somatostatin assay was a gift from J Gerich (Rochester, Minn, USA). It was verified that compound **1f**, when added to the radioimmunoassay incubation medium, did not interfere with the tracer antibody binding. The interassay variation coefficients were 10% (insulin) and 5% (glucagon and somatostatin).

IC_{50} *determination*

For each compound, the IC_{50} value was determined graphically from the corresponding log concentration/insulin secretion inhibition plots. For each determination, at least 4 different inhibitor concentrations were ranged in the linear part of the inhibition curve.

Cytotoxicity studies

Trypan blue exclusion test. Control and test islets were exposed to a 0.5% solution of Trypan blue (w/v) in a 0.154 M NaCl solution for 1 min and then examined under a dissecting microscope (x 40) with episcopic light (Wild M3Z, Heerbrugg, Switzerland).

Statistical analysis. Results are expressed as mean ± SEM. Statistical significance of differences was analyzed by using the student's *t*-test for non-paired values.

References

1 Lemke TL, Shek TW, Cates LA, Smith LK, Cosby LA, Sartorelli AC (1977) *J Med Chem* 20, 1351–1354
2 Shipman C Jr, Smith SH, Drach JC, Klayman DL (1986) *Antiviral Res* 6, 197–222
3 Klayman DL, Bartosevich JF, Griffin TS, Mason CJ, Scovill JP (1979) *J Med Chem* 22, 855–862
4 Mignot A, Miocque M, Binet P, Rapin JR, Rinjard P, Roux M, Cals MJ, Ekindjian JC (1980) *Eur J Med Chem* 15, 33–40
5 Protivinsky R (1971) *Antibiot Chemother (Basel)* 17, 101–121
6 Agrawal KC, Schenkman JB, Denk H, Mooney PD, Moore EC, Wodinsky I, Sartorelli AC (1977) *Cancer Res* 37, 1692–1696
7 Epand RM, Stafford AR, Tyers M, Nieboer E (1985) *Mol Pharmacol* 27, 366–374
8 Osei K, Falks JM, Nelson KP, Stephens R (1984) *Am J Med* 77, 41–46
9 Bany T, Modzelewska-Banachiewicz B, Maliszewska A (1974-1975) *Ann Univ Mariae Curie-Sklodowska, Sect AA* 29/30, 147–152; (1977) *Chem Abstr* 87, 39384z
10 Spassov A, Golovinsky E, Demirov G (1966) *Chem Ber* 99, 3734–3736
11 Voss J, Walter W (1968) *Liebigs Ann Chem* 716, 209–211
12 Patel KS, Mishra RK (1983) *Bull Chem Soc Jpn* 56, 2811–2813
13 Peak DA, Stansfield F (1952) *J Chem Soc* 4067–7075
14 Ün R, Ikizler A (1975) *Chim Acta Turc* 3, 1–22; (1976) *Chem Abstr* 84, 4861p
15 Malbec F, Milcent R, Barbier G (1984) *J Heterocycl Chem* 21, 1689–1698
16 Bellitto C, Gattegno D, Giuliani AM, Bossa M (1976) *J Chem Soc Dalton Trans* 758–762
17 Emdin SO, Dodson GG, Cutfield JM, Cutfield SM (1980) *Diabetologia* 19, 174–182
18 Grodsky GM, Schmid-Formby F (1985) *Endocrinology* 117, 704–710
19 Epand RM (1982) *Mol Pharmacol* 22, 105–108
20 Toropsev IV, Eshchenko VA (1983) *Bull Exp Biol Med* 96, 1215–1217
21 French FA, Blanz EJ Jr (1966) *J Med Chem* 9, 585–589
22 Cristalli G, Franchetti P, Nasini E, Vittori S, Grifantini M, Barzi A, Lepri E, Ripa S (1988) *Eur J Med Chem* 23, 301–305
23 Katritzky AR, Nie PL, Dondoni A, Tassi D (1979) *J Chem Soc Perkin Trans I* 758–762
24 Davidson JS (1979) *Synthesis* 359–361
25 May P (1913) *J Chem Soc* 103, 2272–2275
26 Lacy PE, Kostianovsky M (1967) *Diabetes* 16, 35–39
27 Delaby J, Campana M, Laborie C, Assan R (1989) *Diabète Métab* 15, 123–127
28 Yalow RS, Berson SA (1960) *J Clin Invest* 39, 1157–1166
29 Herbert V, Lau KS, Gottlieb CV, Bleicher S (1965) *J Clin Endocrinol Metab* 25, 1375–1380